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89
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ASPECTS OF PROTEOLYSIS IN CHEESE

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ABSTRACT

The purpose of the present study was to elaborate methods for the detailed examination of proteolysis pathways in cheese (reviewed in Chapter 1) and to demonstrate their usefulness. Many techniques, including solvent fractionation, chromatographic separation and electrophoresis have been used previously and were revisited in this study.

Gel electrophoresis can be a powerful technique and was examined in detail. The methods investigated were: 1) a slab gel system using the apparatus of the E-C Apparatus Corporation and a polyacrylamide gel in a Tris-EDTA-borate buffer at alkaline pH and containing urea; 2) a mini-slab gel system using the Bio-Rad mini-Protean II apparatus, a polyacrylamide stacking and resolving gel with a discontinuous (Tris-chloride/Tris-EDTA-borate) buffer system that contained urea; 3) a mini-slab gel system using the Bio-Rad mini-Protean II apparatus, a polyacrylamide stacking and resolving gel and acetic acid-ammonium acetate buffers at acidic pH that contained urea; 4) a mini-slab gel system using the Bio-Rad mini-Protean II apparatus, a polyacrylamide gel with a stacking and resolving gel in Tris-HCl buffers containing sodium dodecyl sulphate (SDS) and a Tris-chloride-glycine electrode buffer.

The mini-slab alkaline urea polyacrylamide gel electrophoresis (PAGE) method was considered to be the most suitable for monitoring the loss of intact casein during cheese ripening. However, SDS-PAGE gave good resolution of para- κ -casein, β -lactoglobulin and α -lactalbumin and it could therefore be used for the analysis of cheese in which whey proteins have been incorporated or for monitoring the breakdown of para- κ -casein (Chapter 4) in cheese. Two-dimensional PAGE revealed the presence of more bands than were visible using any single method of electrophoresis. Traces of protein were found to lie beneath the α_{s1} -casein band and this explained why, even after considerable proteolysis, some α_{s1} -casein appeared to remain.

Storing cheese samples in such a way that there is a minimum of further change was examined using several different storage methods and temperatures, including storage as: freeze-dried powder at 4°C in the dark, frozen at -9, -16, -35, -75 and -100°C, and dissolved in 6 M urea solution and stored at 4 and -16°C. The trial ran for 6 months and involved the multiple sampling and detailed analysis of three Cheddar cheeses by reversed phase fast protein liquid chromatography (RP-FPLC) for the water-soluble fraction (WSF) and alkaline urea-PAGE for the protein fraction.

None of the methods used to store the cheese samples was completely satisfactory. Cheese stored at temperatures of -9 and -16°C was unstable, with proteolysis discernible after 66 days. Storage of cheese samples at these temperatures is, therefore, not recommended. Cheese stored at temperatures of -35 , -75 and -100°C was unstable after 94 days, although the samples stored at -100°C were more stable. This lack of stability probably arose during thawing as well as during storage of the frozen cheese samples. Storage of freeze-dried samples at 4°C in the dark was equivalent to storing the frozen cheese at -100°C . Storage of samples in alkaline urea sample buffer was better at -16°C than at 4°C but should be for no longer than 1 month.

An indication of the differences to be expected within the normal range of Cheddar cheese was determined using three very similar Cheddar cheeses ripened at 5 and 13°C (Chapter 3, part II). Cheeses ripened at 5°C for 6 months were similar to those ripened at 13°C for 2 months and the proteolytic pathways appeared to be the same at both temperatures.

The proteolytic pathways in Cheddar and Mozzarella cheeses, manufactured according to standard protocols, ripened at 13°C and sampled at regular intervals over a six month period were examined using a variety of techniques: total nitrogen (TN), non-protein nitrogen (NPN), water-soluble nitrogen (WSN), alkaline urea-PAGE, low molecular weight (LMW) SDS-PAGE, RP-FPLC and size exclusion high performance liquid chromatography (SE-HPLC). The TN and NPN analyses were done at the time of sampling whereas the other assays were done on samples that had been stored at $<-75^{\circ}\text{C}$ so that they could be analysed simultaneously.

The increase in WSN and NPN was greater in Cheddar cheese than in Mozzarella cheese and reflected the greater microbial enzyme activity in this cheese type.

Alkaline urea-PAGE revealed that there was more α_{s1} -casein hydrolysis (with the formation of α_{s1} -casein-I) in Cheddar cheese than in Mozzarella cheese, indicating that rennet activity was greater in Cheddar cheese. The presence of peptides believed to be β -I- (β -casein f1-189/192) and β -II-casein (β -casein f1-165) indicated that rennet may have hydrolysed β -casein. The amount of β -casein hydrolysis (and γ -casein formation) was greater in Mozzarella cheese, reflecting the greater plasmin activity in this cheese type. Both LMW SDS-PAGE and SE-HPLC of the whole cheese provided little additional information.

Examination of the WSF of each cheese by PAGE analysis showed that many of the

larger peptides may have been present in both cheese types. The different concentrations of these peptides in each cheese type were consistent with different rennet and plasmin activities and suggested that they may have been products of these enzymes. RP-FPLC and SE-HPLC analysis of the WSF of Cheddar cheese revealed that, although the larger peptides continued to accumulate during ripening, there was also a large increase in the amount of small peptides and amino acids in the cheese. In the Mozzarella cheese, the larger peptides accumulated and there was little evidence of their further hydrolysis to small peptides and amino acids.

The present studies indicate that SE-HPLC using a Toyo-Soda SW 2000 column and a 36% acetonitrile/0.1% trifluoroacetic acid solvent system is a promising new technique that may be useful in determining cheese type and maturity and in relating changes in the molecular weight distribution of the peptides to changes in the textural, functional and flavour characteristics of cheese.

It was concluded that the results are consistent with the concept that differences in the manufacture of Cheddar and Mozzarella cheeses result in the formation of two cheeses, each with different amounts of similar enzymes (rennet, plasmin, and the enzymes of the starter and non-starter lactic acid bacteria), and that these differences in enzyme concentration, combined with the modifying effect of pH, temperature, moisture content and S/M, result in different enzyme activities and patterns of proteolysis in the two types of cheese and these, in turn, result in cheeses with different functional properties.

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TABLE OF CONTENTS

CHAPTER 1 PROTEOLYSIS IN CHEESE: A GENERAL REVIEW

1	INTRODUCTION	12
1.1	CHEESE MANUFACTURE	12
2	THE PROTEOLYTIC ENZYMES IN CHEESE	14
2.1	THE COAGULANT ENZYMES	15
2.1.1	Animal Rennet	15
2.1.2	Recombinant Rennet	15
2.1.3	Microbial Rennet	16
2.1.4	Rennet as a Coagulant	17
2.1.5	Rennet and Proteolysis	20
2.2	PLASMIN	29
2.2.1	Plasmin and Proteolysis of Milk Proteins	29
2.2.2	Plasmin and Proteolysis in Cheese	36
2.3	ENZYMES OF STARTER AND NON-STARTER ORGANISMS	39
2.3.1	Enzymes of Starter Organisms	39
2.3.2	Enzymes of Secondary Microflora	44
2.3.3	Non-starter Lactic Acid Bacteria in Cheese	45
2.3.4	Psychrotrophic Bacteria in Cheese	47
3	TEXTURE AND FLAVOUR DEVELOPMENT IN CHEESE	48
3.1	PROTEOLYSIS AND TEXTURE	50
3.2	PROTEOLYSIS AND FLAVOUR	51
3.2.1	Bitterness in Cheese	53
4	CHEESE VARIETIES	54
4.1	PROTEOLYSIS IN CHEDDAR CHEESE	54
4.2	PROTEOLYSIS IN DUTCH-TYPE CHEESE	56
4.3	PROTEOLYSIS IN SWISS-TYPE CHEESES	58
4.4	PROTEOLYSIS IN MOZZARELLA CHEESE	59
4.5	PROTEOLYSIS IN CAMEMBERT AND BRIE CHEESES	62
4.6	PROTEOLYSIS IN BLUE CHEESE	64
5	CONCLUSION	66
6	BIBLIOGRAPHY	67

CHAPTER 2 GEL ELECTROPHORESIS: A COMPARISON OF METHODS SELECTED TO STUDY PROTEOLYSIS IN CHEESE

1	INTRODUCTION	85
2	LITERATURE REVIEW	87
2.1	GEL ELECTROPHORESIS	87
2.1.1	Polyacrylamide Gel Electrophoresis in Alkaline Conditions	87
2.1.2	Polyacrylamide Gel Electrophoresis in Acid Conditions ...	90
2.1.3	SDS Polyacrylamide Gel Electrophoresis	90
2.1.4	Two-Dimensional Gel Electrophoresis	92
2.2	SAMPLE PREPARATION	93
2.3	STAINING	93
3	OBJECTIVE	95
4	MATERIALS AND METHODS	96
5	RESULTS	104
6	DISCUSSION	115
7	CONCLUSION	117
8	BIBLIOGRAPHY	118

CHAPTER 3 I. OPTIMUM STORAGE CONDITIONS FOR CHEDDAR CHEESE SAMPLES DESTINED FOR QUANTITATIVE PEPTIDE AND PROTEIN ANALYSIS and II. THE EFFECT OF STORAGE TEMPERATURE ON PROTEOLYSIS IN CHEDDAR CHEESE

1	INTRODUCTION	127
2	OBJECTIVES	129
3	EXPERIMENTAL PROCEDURE	130

4	MATERIALS AND METHODS	131
5	RESULTS	134
6	DISCUSSION	152
7	CONCLUSION	158
8	BIBLIOGRAPHY	160

CHAPTER 4 A COMPARISON OF PROTEOLYSIS IN CHEDDAR AND MOZZARELLA CHEESES USING DIFFERENT METHODS OF ANALYSIS

1	INTRODUCTION	165
2	OBJECTIVE	166
3	MATERIALS AND METHODS	167
4	RESULTS	174
5	DISCUSSION	204
6	CONCLUSIONS	214
7	BIBLIOGRAPHY	216

FIGURES

1.1	Breakdown of casein during cheese ripening: involvement of proteinases from various sources	14
1.2	Chymosin action on α_1 -casein	23
1.3	Chymosin action on β -casein	26
1.4	Chymosin action on α_2 -casein	28
1.5	Plasmin action on β -casein	31
1.6	Plasmin action on α_2 -casein	33
1.7	Plasmin action on α_1 -casein	35
1.8	Classes of proteinases	41
1.9	Designation of peptidases	41
1.10	Action of dipeptidyl peptidase and peptidyl peptidase	42
1.11	The interrelationship between proteolytic systems and amino acid transport systems in lactic acid bacteria	42
1.12	Classification of traditionally manufactured cheese varieties by their characteristic ranges of ratio of calcium/solid-non-fat and pH	49
1.13	Diagrammatic representation of the effect of pH and calcium on cheese microstructure and texture	49
1.14	General pathways of amino acid catabolism in cheese	52
1.15	Sequence of proteolysis in Camembert cheese	64
2.1	Polyacrylamide gel electrophoresis patterns of the different cheese varieties on the large alkaline urea (slab) gel	104
2.2	Polyacrylamide gel electrophoresis patterns of the different cheese varieties on the alkaline urea mini-slab gel	105
2.3A	Polyacrylamide gel electrophoresis patterns of the different cheese varieties on the acid urea mini-slab gel	106
2.3B	Acid urea polyacrylamide gel electrophoresis. Acid versus alkaline urea sample buffer. Mercaptoethanol versus no mercaptoethanol	109
2.4	Two-dimensional polyacrylamide gel electrophoresis. "Mature" Cheddar cheese electrophoresed on an alkaline urea gel for the first dimension and an acid urea gel for the second dimension	110
2.5	Two-dimensional polyacrylamide gel electrophoresis. Feta cheese sample electrophoresed on an alkaline urea gel for the first dimension and an acid urea gel for the second dimension	111
2.6	Polyacrylamide gel electrophoresis patterns of the different cheese varieties on the "low molecular weight" SDS mini-slab gel	112

2.7	Two-dimensional polyacrylamide gel electrophoresis. "Mature" Cheddar cheese electrophoresed on an alkaline urea gel for the first dimension and a LMW SDS gel for the second dimension	113
2.8	Two-dimensional polyacrylamide gel electrophoresis. Feta cheese electrophoresed on an alkaline urea gel for the first dimension and a LMW SDS gel for the second dimension	114
3.1	The effect of storage temperature (-100 to -9°C) on the ratio of α_1 - to β -casein in Cheddar cheese from vats 1, 2 and 3	135
3.2	The effect of storage temperature (-100 to -9°C) on the ratio of α_1 -casein to α_1 -casein-I in Cheddar cheese from vats 1, 2 and 3	136
3.3	RP-FPLC profile of a mixture of amino acids. The absorbance was monitored at 214 and 280 nm	138
3.4	RP-FPLC. The effect of storage temperature on the peptide profiles of the WSF of Cheddar cheese from vats 1, 2 and 3	139
3.5	The effect of storage temperature (-100 to -9°C) on the amount of water-soluble peptide and amino acid material with an absorbance at 214 nm	140
3.6	RP-FPLC. The effect of storage temperature (-100 to -9°C) on the amount of material, that eluted between the tryptophan peak and 75 min	141
3.7	The effect of storage time on the ratio of α_1 - to β -casein in freeze-dried Cheddar cheese samples from vats 1, 2 and 3	143
3.8	The effect of storage of freeze-dried cheese samples on the peptide profiles obtained by RP-FPLC analysis of the WSF of Cheddar cheese from vats 1, 2 and 3 . . .	144
3.9	The effect of storage of Cheddar cheese samples from vats 1, 2 and 3 in alkaline urea sample buffer on the urea-PAGE patterns of the caseins	145
3.10	The effect of storage of Cheddar cheese samples from vats 1, 2 and 3 in alkaline urea sample buffer on the apparent ratio of α_1 - to β -casein	146
3.11	The effect of ripening temperature on proteolysis in Cheddar cheese from vats 1, 2 and 3	149
3.12	The effect of ripening temperature on the RP-FPLC profiles of the WSF of Cheddar cheese from vats 1, 2 and 3	150
4.1	Changes in WSN and NPN (expressed as a percentage of the TN) in Cheddar and Mozzarella cheeses during maturation at 13°C	176
4.2	Alkaline urea-PAGE. Casein degradation in (A) Cheddar cheese and (B) Mozzarella cheese ripened at 13°C	178
4.3	Trends in α_1 - and β -casein breakdown in Cheddar and Mozzarella cheeses ripened at 13°C, and sampled after 1, 14, 28, 63, 91, 133 and 182 days	179

4.4	Proteolysis monitored by LMW SDS-PAGE in (A) Cheddar cheese and (B) Mozzarella cheese ripened at 13°C	181
4.5	The increase in dye intensity in the region of para- κ -casein in Cheddar and Mozzarella cheeses ripened at 13°C	182
4.6	Alkaline urea-PAGE. The water-insoluble fraction of (A) Cheddar cheese and (B) Mozzarella cheese stored at 13°C	184
4.7	LMW SDS-PAGE. The water-insoluble fraction of (A) Cheddar cheese and (B) Mozzarella cheese stored at 13°C	185
4.8	Alkaline urea-PAGE. The WSF of (A) Cheddar cheese and (B) Mozzarella cheese stored at 13°C	188
4.9	LMW SDS-PAGE. The WSF of (A) Cheddar cheese and (B) Mozzarella cheese stored at 13°C	190
4.10	RP-FPLC of the WSF of Cheddar and Mozzarella cheeses after 1, 14, 28, 63, 91, 133 and 182 days maturation	194
4.11	RP-FPLC. The total accumulated peak area, measured at 214 and 280 nm, for Cheddar and Mozzarella cheeses after 1, 14, 28, 63, 91, 133 and 182 days	195
4.12	RP-FPLC. The absorbance at 214 nm of three peaks corresponding to tyrosine, phenylalanine and tryptophan in Cheddar and Mozzarella cheeses	196
4.13	SE-HPLC calibration curve of standards	198
4.14	SE-HPLC of the urea-soluble fraction of Cheddar and Mozzarella cheeses . . .	200
4.15	SE-HPLC of the WSF of Cheddar and Mozzarella cheeses	203

TABLES

3.1	RP-FPLC retention times of amino acid standards	137
3.2	The effect of freezing at different temperatures and freeze-drying on the pH of milk, cheese whey, alkaline urea sample buffer and the WSF of cheese	147
3.3	The effect of storage temperature (5 and 13°C) on the amount of material that eluted between tryptophan and 75 min	151
4.1	The compositional analyses of the Cheddar and Mozzarella cheeses	174
4.2	Alkaline urea-PAGE. The relative mobilities of the bands in the WSF of Cheddar and Mozzarella cheeses	189
4.3	LMW SDS-PAGE. The relative mobilities of the bands in the WSF of Cheddar and Mozzarella cheeses	191